Regulation of adiponectin multimerization, signaling and function

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Adiponectin, which exists in serum in three major complexes including trimer, hexamer, and the high molecular weight (HMW) form, has strong insulin sensitizing, anti-inflammatory and anti-diabetic functions. Different adiponectin complexes exert tissue-specific biological functions and activate distinct signaling pathways. In this review, we summarize our current understanding on the mechanisms regulating adiponectin multimerization. We also describe the major target tissues in which distinct adiponectin multimers exert their functional roles. Finally, we discuss the potential involvement of endoplasmic reticulum stress and mitochondrial stress in diet-induced adiponectin downregulation and highlight the roles of Disulfide bond A oxidoreductase-like protein (DsbA-L) in the prevention of endoplasmic reticulum stress and promotion of adiponectin multimerization, stability, and function.

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Introduction

Adiponectin has great potential as a therapeutic target for a variety of obesity-associated diseases, including type 2 diabetes, non-alcoholic steatotic hepatitis (NASH), and atherosclerosis\textsuperscript{[1–4]}. However, manipulation of circulating adiponectin has been quite challenging due to its complicated multimeric structure and the presence of high concentrations of this adipokine in serum, which is roughly 3 orders
of magnitude greater than most other hormones in humans [5]. The successful development of adiponectin as an effective therapeutic drug, therefore, is critically dependent upon our understanding of the cellular mechanisms regulating adiponectin biosynthesis, secretion, and action.

Adiponectin circulating in plasma exists in three major forms: trimer, hexamer, and high molecular weight (HMW) multimer [6–9], as well as a proteolytically cleaved form, globular adiponectin [10,11]. The percentage of the HMW and hexamer multimers was relatively higher in the plasma of mice than in human subjects [12,13]. Different adiponectin multimers, which have been shown to exert distinct biological properties, do not interconvert once in circulation [13]. The HMW form of adiponectin has been shown to be the more active form of the protein and has a more relevant role in improving insulin sensitivity and protecting against diabetes [14–17]. Impaired adiponectin multimerization, particularly selective reduction of the HMW adiponectin concentrations, was found to be associated with various metabolic diseases such as obesity, insulin resistance, type 2 diabetes, and arteriosclerosis [5,17–20] and contributed to the worsening of insulin resistance and its metabolic complications in myotonic dystrophy type 1 patients [21]. An increase in the HMW form, rather than the total level of adiponectin, was found to be associated with insulin-sensitizing effect of Thiazolidinediones (TZDs) in both mice and human diabetic patients [20]. Consistent with this finding, increasing the HMW form of adiponectin by fat-specific overexpression of DsbA-L protected mice from diet-induced insulin resistance [12]. In fact, it has been suggested that reduced levels of the HMW form rather than total levels of adiponectin could be a superior biomarker for insulin resistance, the metabolic syndrome, and type 2 diabetes [17]. However, how adiponectin multimerization is regulated and whether impaired adiponectin multimerization has a causative role in insulin resistance and metabolic dysfunction remains largely unclear, which greatly hinders the development of adiponectin-based therapeutic treatments. This review describes the most recent advances in the understanding of the mechanisms regulating adiponectin biosynthesis and function.

**Molecular structure and multimerization of adiponectin**

Adiponectin, a member of the complement 1q family, consists of a signal sequence at the N-terminus, followed by a variable region, a collagenous domain, and a C-terminal globular domain [22,23]. A number of studies have demonstrated that the N-terminal region of adiponectin plays an essential role in the multimerization of this adipokine. There is a conserved cysteine residue at the N-terminus (Cys^36 in human and Cys^39 in mouse) and the formation of an intermolecular disulfide bond via this residue is essential for adiponectin multimerization and secretion [8]. Replacing Cys^39 with serine completely disrupted the assembly and secretion of hexamer and HMW adiponectin, but had very little effect on the formation of the trimer [8]. Cys^39 was also identified as a key site of succination of adiponectin which blocks adiponectin multimerization, and may contribute to the decrease in plasma adiponectin in diabetes [24]. Another highly conserved amino acid residue within the N-terminus is tryptophan (W^42) and a mutation of this residue profoundly affects adiponectin assembly by trapping full-length adiponectin in the oxidized trimeric or hexameric states, probably due to a proximity effect causing a reduction in the rate of Cys^39 oxidation [25]. In addition to disulfide bond formation, adiponectin multimerization is also regulated by hydroxylation and glycosylation [26]. There are four conserved lysine residues in the collagenous domain of adiponectin (Lys^65, Lys^68, Lys^77, and Lys^101 for human adiponectin) and hydroxylation and subsequent glycosylation at these sites are required for intracellular assembly of the trimer of adiponectin into the HMW multimer [26–28]. Hydroxylation is also detected at several proline residues in human adiponectin including Pro^71, Pro^76, and Pro^95. Inhibition of proline hydroxylation results in a more severe impairment of adiponectin multimerization, although the physiological role of this hydroxylation remains to be further clarified [28]. A number of mutations have been identified in human adiponectin that are associated with impaired adiponectin multimerization and metabolic diseases [8]. The G84R and G90S mutants of adiponectin do not form HMW multimers and are associated with diabetes and hypoadiponectinemia [8]. R112C and I164T mutants, which are associated with hypoadiponectinemia, fail to assemble into trimers, resulting in impaired secretion from the cell [8]. These data provide evidence for a link between impaired adiponectin multimerization and the causes of a diabetic phenotype in humans and suggest that not only total concentrations, but also multimer distribution should always be a consideration in the interpretation of plasma adiponectin levels in health as well as various disease states.
Adiponectin stability and endoplasmic reticulum (ER) stress

It is well documented that the cellular and serum levels of adiponectin are negatively correlated with obesity [14–17]. The precise underlying mechanisms, however, remain elusive. Our recent studies demonstrate that obesity-induced endoplasmic reticulum (ER) stress may play a causative role in obesity-induced adiponectin downregulation [29]. ER is a eukaryotic organelle responsible for several specialized and important functions such as protein translation, folding, and transportation of membrane or secreted proteins. Numerous protein chaperones are present in the ER lumen that yield an oxidizing environment necessary for correct folding and assembly of various membrane and secretory proteins such as adiponectin. In the obese state, an increased demand on ER function causes ER stress which triggers the unfolded protein response (UPR) to remove incorrectly folded proteins so that normal cell function and viability are maintained [30]. We have found that inducing ER stress by thapsigargin, a chemical widely used to stimulate ER stress by inhibiting the ER calcium-ATPase [31], was sufficient to reduce adiponectin levels in 3T3-L1 adipocytes [29]. In addition, inhibiting ER stress by the chemical chaperone tauroursodeoxycholic acid (TUDCA) partially restored adiponectin levels in db/db mice and diet-induced obese mice [29]. The ER stress-induced adiponectin downregulation, which is mediated by an autophagy-dependent mechanism [29,32], could be protected by overexpression of the disulfide bond oxidoreductase A-like protein (DsbA-L) [33], an adiponectin-interactive protein that selectively promotes adiponectin multimerization in adipocytes [34] and in vivo [35]. Overexpression of DsbA-L reduced high fat diet-induced ER stress and increases adiponectin multimerization as well as total levels of adiponectin in mice [35]. Taken together, these findings suggest a causative effect of ER stress in obesity-induced adiponectin downregulation. The precise mechanisms by which DsbA-L improves ER stress and promotes adiponectin multimerization remain unknown, but the protein may function as a chaperone to facilitate correct folding and assembly of adiponectin and potentially other macromolecules in the ER. In addition to DsbA-L, several other ER chaperons, including the ER membrane-associated oxidoreductase Ero1-La and its associated protein ERp44, have also been identified as critical players in the assembly of higher-order adiponectin complexes and secretion [9,36].

Mitochondrial function and adiponectin biosynthesis

In addition to the ER, some recent studies suggest a potential link between mitochondrial dysfunction and impairment in adiponectin biosynthesis, stability, and function. Mitochondrial copy number and the expression of adiponectin are both reduced in db/db mice, but could be reversed by the anti-diabetic agent rosiglitazone [37]. Stimulation of mitochondrial biogenesis by overexpressing nuclear respiratory factor-1 also leads to enhanced adiponectin expression levels [37]. On the other hand, increased intracellular reactive Oxygen species (ROS) levels elicited by mitochondrial dysfunction resulted in impairment of insulin action and adiponectin secretion in adipocytes [38]. Very recently, it has been shown that mitochondrial dysfunction-induced ROS overproduction is associated with decreased levels of the HMW form of adiponectin in adipose tissues of the caveolin-1 knockout mice [39]. In addition, enhancing mitochondrial function promoted adiponectin secretion [40]. Taken together, these results provide further evidence on a link between mitochondrial function and adiponectin biosynthesis. However, how mitochondrial dysfunction leads to the impairment of adiponectin biosynthesis, secretion and stability remains unknown. Interestingly, mitochondrial dysfunction has been shown to increase ER stress [37], providing a mechanistic link between mitochondrial dysfunction, ER stress, and adiponectin downregulation [32].

The targeted tissues and signaling mechanisms underlying the action of adiponectin multimers

Liver has been shown to be the major adiponectin target tissue and play a critical role in the insulin sensitizing effect of adiponectin [16,41–45]. By targeting to the liver, the full-length adiponectin, which circulates in trimeric, hexameric and higher order complexes [6–9], activated AMP-activated protein kinase (AMPK) and reduced the expression of gluconeogenic enzymes such as phosphoenolpyruvate carboxylase and glucose-6-phosphatase, leading to the suppression of gluconeogenesis [43,45].
Consistent with these results, we found that increasing the HMW form of adiponectin by fat-specific overexpression of DsbA-L enhanced AMPK phosphorylation at Thr\textsuperscript{172} in the liver but not in the skeletal muscle, concurrent with increased resistance to diet-induced obesity, insulin resistance and hepatosteatosis [35].

The signaling pathways mediating the insulin sensitizing effects of adiponectin in the liver remain to be fully characterized. An earlier study showed that suppressing the expression levels of AMPK in liver by adenovirus-mediated overexpression of dominant-negative AMPK partially but significantly reduced the glucose-lowering effect of adiponectin, suggesting that activation of AMPK is critical for adiponectin action in the liver [16]. However, adiponectin has later been found to inhibit gluconeogenic gene expression and suppress hepatic glucose production (HGP) in primary mouse hepatocytes lacking AMPK or the AMPK upstream kinase, liver kinase B1 (LKB1) and in mice in which the lkb-1 gene is targeted for deletion in the liver [46], suggesting that AMPK is dispensable for the glucose lowering effect of adiponectin in the liver. Consistent with these results, full-length adiponectin has been shown to suppress HGP by up regulation of hepatic insulin receptor substrate 2 (IRS-2) via an interleukin 6 (IL-6)-dependent but AMPK-independent pathway [47]. However, how adiponectin suppresses HGP via an AMPK-independent mechanism remains unknown. A recent study showed that adiponectin was able to enhance the ceramidase activity of its receptors including adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2), leading to a reduction in hepatic ceramide content and improved hepatic and whole-body insulin sensitivity independent of AMPK [48]. Another possible mechanism by which adiponectin exerts its functions via an AMPK-independent pathway is through modulating the biological actions of growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) [49]. The binding of adiponectin to these growth factors is oligomerization-dependent. PDGF-BB binds to the HMW and middle molecular weight (MMW, equivalent to the hexameric form) complexes, and basic FGF preferentially interacts with the HMW form, whereas HB-EGF binds to all three forms with comparable affinities [49].

The globular form of adiponectin (gAd) has been shown to activate the AMPK pathway in skeletal muscle, leading to increased phosphorylation of coenzyme A carboxylase (ACC), fatty acid oxidation and glucose uptake [14,16]. Incubation of rat extensor digitorum longus (EDL), a predominantly fast twitch muscle, with gAd but not the full-length adiponectin, increased AMPK activity and phosphorylation of both AMPK and ACC [14]. The effect of adiponectin in skeletal muscle is inhibited by suppressing the expression levels of the adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif (APPL1), an AdipoR1 and AdipoR2 interactive protein that positively mediated adiponectin signaling and its insulin sensitizing effect in muscle cells [50–54]. In addition to mediating adiponectin in muscle cells, APPL1 is also found to play a key role in adiponectin signaling and action in many other cells including, human cardiac microvascular endothelial cells [55], cardiomyocytes [56], human THP-1 monocytes [57], H9c2 cardiomyocytes [58], and RAW264.7 macrophage cells [56], as well as in the heart [56], and in the hypothalamus [59].

Besides functioning in peripheral tissues, adiponectin has also been found to act in the central nervous system (CNS) to regulate appetite and energy expenditure. Both AdipoR1 and AdipoR2 are detected in the paraventricular hypothalamic area (PVH) [60], the arcuate (Arc) and lateral hypothalamic (LH) nuclei [59], suggesting a physiological involvement of adiponectin action in these brain regions. Consistent with this, Intracerebroventricular (i.c.v.) injection of adiponectin in rats has been found to induce the associations of AdipoR1 and AdipoR2 with APPL1, leading to activation of adiponectin receptor downstream signaling in the hypothalamus [59]. Kubota et al. [60] showed that the LMW (trimeric) adiponectin, which can pass the blood brain barrier and enter into the cerebrospinal fluid from circulation, plays a major role in regulating feeding behavior in the CNS. However, there have been some controversies on whether adiponectin stimulates, suppresses, or has any role in food intake. Kubota et al. [60] have shown that by binding to AdipoR1 in the arcuate hypothalamus (ARH), adiponectin stimulates AMPK activity and increases food intake. Consistent with this finding, adiponectin deficiency led to a lean phenotype with reduced food intake and increased energy expenditure [60]. On the other hand, intracerebroventricular (i.c.v) infusion of adiponectin has been found to suppress food intake in mice [59] and rats [61]. There is also a report showing that i.c.v. administration of adiponectin in mice, while resulting in decreased body weight and increased energy expenditure, had no significant effect on food intake [62]. The precise reason for these discrepancies remains unknown but distinct
roles of acute and chronic i.c.v. injection of adiponectin have been suggested [61]. A recent study has also suggested an antidepressant-like activity of adiponectin in the brain. In support of this view, icv injection of an adiponectin neutralizing antibody precipitated stress-induced depressive-like behavior [63]. Conversely, icv administration of exogenous adiponectin produced antidepressant-like behavioral effects in normal-weight mice and in diet-induced obese mice [63].

Adiponectin upregulates IRS-2 through activation of signal transducer and activator of transcription-3 (STAT3) [47]. This stimulation, which is associated with increased IL-6 production from macrophages through NFkB activation, is mediated by an uncharacterized receptor independent of AdipoR1 and AdipoR2 [47]. While the identity of this receptor is currently unknown, the stimulatory effect of adiponectin on IL-6 production in RAW264.7 cells has been found to be isoform-dependent: the full-length adiponectin with all three multimers has the most potent effect compared with the trimeric form alone, whereas globular adiponectin did not induce IL-6 at all [47]. These results provide further evidence on the distinct roles of individual adiponectin multimers in regulating cell signaling events.

Summary

Adiponectin circulates in serum in the forms of trimer, hexamer and the HMW multimer and different adiponectin multimers have been shown to exert distinct biological functions. Through activation of AMPK-dependent and -independent pathways, the full-length adiponectin functions as an insulin sensitizer mainly in the liver to suppress glucose production. The proteolytically processed globular form of adiponectin, on the other hand, activates AMPK and promotes fatty acid oxidation mainly in skeletal muscle to promote glucose uptake. In addition to targeting peripheral tissues, the hexamer and trimer forms of adiponectin also act in the central nervous system (CNS) to regulate appetite and energy expenditure as well as to produce antidepressant-like effects. The multimerization and total levels of adiponectin are suppressed in obesity. On the other hand, alleviating ER stress by increasing the cellular levels of ER chaperons such as DsbA-L can prevent diet-induced and ER stress-mediated adiponectin down-regulation. This sheds light on some promising strategies to combat obesity-induced insulin resistance and associated metabolic diseases.

Practice points

- Different adiponectin multimers exert distinct biological properties and tissue-selective functions.
- Impaired adiponectin multimerization is associated with various metabolic diseases, such as obesity, insulin resistance, type 2 diabetes, and arteriosclerosis.
- DsbA-L promotes adiponectin multimerization and prevents adiponectin from diet-induced and ER stress-mediated downregulation.

Research agenda

- How DsbA-L promotes adiponectin multimerization?
- Is there a functional link between ER and mitochondria in regulating adiponectin biosynthesis and secretion?
- Is promoting adiponectin multimerization by small molecules a promising strategy to combat obesity-induced insulin resistance and associated metabolic diseases?

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